AN INVESTIGATION INTO THE POOR EMERGENCE OF *Pinus elliottii* AND *Pinus taeda* SEEDS.

Honours Project of **MELANIE ASARY**
Supervisor : **Dr. J. M. Farrant**
Botany Department
University of Cape Town

ABSTRACT

The pine seeds *Pinus elliottii* and *Pinus taeda* have a low percentage germination in the MONDI nurseries. This study investigates the effect of different dormancy-breaking treatments on the germinability of the seeds. Germination tests performed showed that these seeds responded positively to these treatments, but the percentage germination was low. Ultrastructural studies reveal that a proportion of the seeds were damaged. This is suggested to be due to the seeds being harvested immature (picked at an early stage of development), inappropriate postharvest practices and fungal infection enhanced upon (possibly) sub-optimal storage conditions.

INTRODUCTION

The successful growth of a seed is important not only biologically, but also economically. Biologically, a mature seed is the means by which a new individual - a plant - is formed. People depend on the successful growth of the seed for almost all their utilisation of plants. These plants are primarily used as food sources for the maintenance of life. However, the use of trees in paper-making (and other by-products) could be of secondary importance in everyday life.

A South African based commercial paper-making company, MONDI, uses pine trees to aid in making paper. In this industry, elite clones are propagated by cuttings or grafting. However, propagation by seed is still of importance to the industry. Plants grown from seed are used as root stocks for grafting. Seeds are also a source of genetic variation (as opposed to cuttings which propagate the same gene pool) which is important to maintain for many reasons (Smith and Berjak, 1995). The trees *Pinus elliotii* and *Pinus taeda* are important in the paper-making process. For economic purposes it is preferable to the industry that the seeds of these trees have a high percentage germination. However, previous experiments showed that these seeds have low germination percentages (Bayley and Rosser, 1992, unpublished). These researchers used cold stratification, various priming solutions, stacking temperatures and scarification, in an attempt to improve the germinability of the seeds, but the results showed that the germinability was still low. It has also been reported that improved germination occurred when the seeds were grown under conditions of high

humidity and heat (Blakeway, pers comm.). We hypothesise that there are two possible reasons for the low germinability : the seeds are dormant seeds or of a low quality.

Dormant seeds are unable to germinate despite conditions being conducive for germination. Many authors define dormancy differently (Bewley and Black, 1994; Brown, 1993; Hillhorst, 1988). However in a review of these authors definition of dormancy, Vleeshouwers *et al.* (1995) attempted to provide an overall definition of dormancy and defined it as a seed characteristic, the degree of which defines what conditions should be met to make the seed germinate. Seeds may evolve certain dormancy breaking strategies that are suited for their environment. Dormant seeds usually need a cue to germinate. An example of this would be pine seeds, which germinate in cold regions and thus would require below-zero temperatures as a dormancy breaking cue (Bewley and Black, 1994). In this experiment, dormancy breaking treatments (which might be relevant to pine and other seeds types) will be used to test the germinability of the pine seedlings *P. taeda* and *P. elliottii*. These treatments are red light, smoke and hormones.

In forest species, orange / red region of the spectrum are often most effective in breaking dormancy (Bewley and Black, 1994). Light is absorbed by molecules of a pigment called phytochrome (Hart, 1988) which exists in two forms, the dormancy-inducing form (Pr) and the dormancy-breaking form (Pfr). When Pr is present in the unirradiated dormant seed it is unable to break dormancy. If the seed is exposed to 660nm of light, it can be changed into Pfr. The active form is able to absorb 730nm of light and is the dormancy-breaking form. In general, germination is positively correlated with Pr content, any situation which reduces the level of Pr lowers the percentage germination (Hart, 1988). Furthermore, Hart (1988) stated that the sensitivity of seeds to light is directly related to its water content. Dry seeds at a moisture content of around 8% are less sensitive to light. However, Pr-Pfr reversibility of response is usually apparent at a moisture content of around 20% (Hart, 1988) - i.e. at a point well before full imbibition. A red light environment can be simulated in the laboratory by exposing seeds to red light filters. We exposed both dry seeds and seeds that have been imbibed for twenty-four hours, to red light treatments. If this shows an

increase in the germinability of the seeds, MONDI could expose the seeds to red light environment via red light filters.

Smoke treatment as a possible germination cue for the *P. taeda* and *P. elliottii* seedlings was chosen because of its success in releasing dormancy in a number of fynbos seeds (Brown, 1993) and non-fybos species (van Staden *et al.* 1995a). Van Staden *et al.* (1995b) suggested that the volatile stimulants in smoke could be involved in breaking different types of seed dormancy. Fynbos species germinate after fires and Brown (1993) proposed that the factors responsible for the germination of the seeds could be : heat of fracturing hard seed coats, heat stimulating seed embryos to germinate, high temperature desiccation of seed coats, ethylene and ammonia in smoke stimulating germination and unknown chemical factors in plant-derived smoke and smoke extracts stimulating germination. Dixon *et al.* (1995) showed that the exposure of dormant seed to cold smoke treatment, had a positive influence on germination on 45 out of 94 species of native Western Australian plants that are hard to germinate. However, to date no smoke treatment experiments have been performed on *P. taeda* and *P. elliottii* seedlings to test the germinability of the seedlings. If smoke treatment does stimulate germination in the two seed types, it would be possible for MONDI to burn pine bark, in this way exposing the seedlings to smoke.

Hormones play an important role in the growth and regulation of seeds during seed development and germination (Bewley and Black, 1994). The effect of gibberillins (GA_3), auxins (IAA) and 6-benzylaminopurine (BAP) will be the principle hormones used to test germinability in this study.

Developing seeds are rich in GA's (Bewley and Black, 1994). Hilhorst and Karssen (1988) showed that the interaction of light and different concentrations of gibberillic acid stimulated the germination of two related seed species *Sisymbrium officinale* and *Arabidopsis thaliana*. Furthermore, Hilhorst (1993) cited in Vleeshouwers *et al.* (1995) presented a model which explained that the stimulation of germination in dormant seeds was accomplished by the interaction of a phytochrome-receptor complex with GA. The major auxin in developing seeds is indolacetic acid (IAA). The

effect of IAA and BAP have been shown to stimulate germination of seedlings in the date species, *Phoenix dactylifera* var *Deglet Nour* (Ammar *et al.* 1987). The exogenous application of selected hormones to the pre-treated *P.taeda* and *P. elliottii* seeds will be accomplished by tissue culture techniques. If the exposure of the seeds to the different hormones (via the tissue culture media) stimulates germination , MONDI could use the technique in their laboratories.

Another possible cause of the low germinibility in the seeds could be that the seeds are of low quality due to damage caused by seed storage practices. Seed storage has been a practice for decades. Throughout this time it has been realised that two important aspects of storage - retention of quality and minimisation of losses, can be realised under the correct conditions (Mycock and Berjak, 1995). However, Mycock and Berjak (1995) state that when harvested seed is placed in commercial storage, the conditions are usually sub-optimal and the seeds are not uniformly sound (which could be due to pre and post harvest conditions).

Orthodox seeds are storable seeds that are desiccation tolerant (Roberts, 1973). The viability of these seeds under certain storage conditions are determined, primarily, by the interrelationships between the moisture content and temperature at which these seeds are stored, as well as the time period over which the seeds are stored (Bewley and Black, 1994). However, the pre and post harvest conditions can also play a role in determining the viability of the seeds (Bewley and Black, 1994). For instance, during harvest the seed can be damaged by e.g. reaping and threshing (Christensen and Kaufmann, 1974). Postharvest drying can cause cracking of the seeds and exposure to fungal infection (Mycock and Berjak, 1995).

Although the chances of fungal attack on the stored seed is increased when the seed is damaged, Mycock and Berjak (1995) states that a seed is subject to invasion from both internally and externally located fungi throughout its existence. A seed is a rich source of food for micro-organisms and fungal infection can cause damage to the embryo, thereby reducing seed vigour and viability. So, in order to minimise fungal infection during seed storage, the storage conditions of the seed types should be optimised.

Even under optimal storage conditions seeds could degenerate due to the ageing of the stored seed. As the storage time increases, the composition of the fungal flora associated with seed tissues changes (Mycock and Berjak, 1992). A loss of integrity of the plasmalemma and tonoplast in aged seeds is implied from observations that more substances leak into the imbibition medium from such seeds than from unaged seeds (Parrish and Leopold, 1978). Furthermore, Bewley (1986) states that increased leakage of organic metabolites could have an indirect, secondary damaging effect by encouraging growth of contaminating micro-organisms on the seed surface. This is supported by Mycock and Berjak (1995), who stated that seed ageing can result in a loss of membrane integrity leading to leakage of biomolecules which could lead to the stimulation of fungal growth. In order to test for seed damage, the electrolyte leakage of the seeds of *P. taeda* and *P. elliottii* was tested. Transmission electron microscopy (TEM) studies were performed to assess the ultrastructural status of the seeds. If the high rate of leakage is due to damaged seeds, we would advise MONDI to improve their harvest and /or seed storage practices.

The aim of this project is to test the germinability of the pine seedlings *P.taeda* and *P.elliottii* using the above treatments. Firstly, to assess whether dormancy is the cause of the low germinability in the seedlings, the seedlings will be exposed to:

a) Red light treatment
b) Smoke treatment
c) Tissue Culture techniques

Secondly, to assess whether the low germinability is due to the seeds being damaged, the conductivity of the seeds will be measured. Finally, the ultrastructural status of the seeds will be examined to determine whether the low germinability of the seeds is due to seed ageing or damage during seed storage.

MATERIALS AND METHODS

The germination treatments were performed on the following seeds:

1) Preliminary experiments were performed on a 1994 seed lot *P. taeda* and *P.elliottii* seeds supplied from Mondi, Pietermaritzburg.
2) 1995 *P. elliotti* seeds from supplied from Mondi Pietermaritzburg.
3) *P . elliottii* seeds, suspected of being damaged.

The treatments as described below were performed on all the above seed lots or combinations of the seed lots.

TREATMENTS

Seeds were subjected to various procedures described below. Control seeds were surface sterilised, imbibed and the viability was determined as described for treated seeds. Prior to treatment, the seeds were divided into two batches. The first batch was imbibed for 24 hours prior to subsequent treatment. The second batch were treated dry. After treatment the viability was assessed by determining the amount of electrolyte leakage over a period of one hour (described below). These seeds were then set out to germinate on filter paper in petri dishes maintained in the dark at 30°C. Each treatment was repeated twice. In the first instance seeds were watered with distilled water. In the second, they were watered with a solution of 0.05% benlate.

1. Red light Experiment

1.1) Non-benlate red light experiment

Dry and imbibed seeds of *P. taeda* and *P. elliottii* were surface sterilised (33% sodium hypochlorite for 10 minutes, rinsed in distilled water twice), exposed to red light 24 hours, and the conductivity determined. Five replicates of ten seeds each were set out to germinate for 20 days on filter paper in petri dishes, in a chamber set at 30°C. The petri dishes were sealed with Whatman parafilm and watered with distilled water when necessary.

1.2 Benlate Red light experiment

The 1995 seed lot and the 'damaged' seed lot were surface sterilised (0.05% ethanol for 1 min., 33% sodium hypochlorite for ten minutes., rinsed three times in distilled

water and finally, rinsed twice in autoclaved water), exposed to red light treatment for 24 hours and the conductivity reading was determined. Ten replicates of ten seeds were set out to germinate on autoclaved filter paper, in petri dishes, in a chamber set at 30°C. The petri dishes were sealed with Whatman parafilm and watered with 0.05% benlate when necessary.

1.2) Smoke Experiment

Both the 1994 and the 1995 seed lots were smoke treated. Five paper discs (Whatman no.5 filter paper with primer) of Instant Smoke Plus Seed Primer (National Botanical Institute, Cape Town) were soaked in 50ml of distilled water for ten minutes. After disposing of the filter paper, the dry and imbibed seeds were soaked in the primer solution for 24 hours. The seeds were surface sterilised. The 1994 seed lot was surface sterilised in 33% sodium hypochlorite for 10 minutes and rinsed in distilled water three times, while the 1995 seed lot was surface sterilised in 0.05% ethanol for 1 min., 33% sodium hypochlorite for ten minutes., rinsed three times in distilled water and finally rinsed twice in autoclaved water. The conductivity was determined after surface sterilisation. For each of the dry and imbibed seeds, ten replicates of ten seeds each (for the 1995 seed lot) and five replicates of ten seeds each (for the 1994 seed lot) were germinated for 20 days on autoclaved filter paper in petri dishes, in a chamber set at 30°C. The petri dishes were sealed with Whatman parafilm and watered with 0.05% benlate when necessary.

2. Tissue Culture

The general culture medium used consisted of a full strength Murashige and Skoog (1962) (MS) medium with the addition of 5% sucrose, 2,5% agar, 1mg / ml 6-Benzylaminopurine (BAP), 1mg /ml Naphthalene Acetic Acid (IAA) and 1mg /ml Gibberillic acid (GA_3). The seeds (25 per treatment) were surface sterilised (0.05% ethanol for 1 minute, rinsed three times with distilled water, 33% sodium hypochlorite for 10 minutes and rinsed 3 times with autoclaved distilled water), after which the seeds were plated by dipping an autoclaved forceps into ethanol, flaming the forceps and upon cooling, transferring the seeds into the autoclaved culture medium. The seeds were grown on a medium with the addition of the following combination of hormones

a) no hormones, b) BAP c) BAP + IAA and d) BAP + IAA + GA_3. The cultures were maintained at 25°C with a 12 / 12 hour light / dark period.

3. Conductivity Experiment

A Jenway hand conductivity meter was used, in which the conductivity of 50 combined seeds was determined. Seeds were placed into a beaker filled with 50ml millipore purified water and the conductivity of the solution was determined every 10 minutes for one hour. Leakage rate was determined from the slope of the regression and was corrected for seed fresh weight.

4. Germination Calculations

Seeds were considered to have germinated once the radical had protruded to a length of 5mm. The final percentage germination was determined after 20 days. Germination rate was calculated according to Czabater (1960) as :

Maximum value obtained from $\left[\%\ \textbf{Germination on day}\ x \div \textbf{day}\ x\right]$.

5. Electron Microscopy

The radical tips of the untreated embryos from each seed lot were fixed overnight 2.5% gluteraldehyde (buffered in 0.1M phosphate of pH 7.4 containing 0.5% caffeine). After three successive washes (in caffeinated phosphate buffer), they were post fixed for 1 hour in 1% osmium tetroxide, and again washed three times in buffer. Samples were then dehydrated using an ethanol series (30, 50, 75, 80, 90 and 100%) at room temperature. The final dehydration involved two changes of 100% alcohol for 10 minutes each. Material were placed into two changes of 100% acetone (10 minutes each), before being placed into 50% resin, 50% acetone solution for 4 hours, followed by their placement in 100% resin overnight. Polymerisation took place for 16 hours at 60°C. The embedded samples were sectioned on a Reichert Ultracut - S (Leica) ultramicrotome stained in uranyl acetate and lead citrate (Reynolds, 1963), and examined in a JEOL200CX transmission electron microscope.

RESULTS

EFFECT OF RED LIGHT AND SMOKE TREATMENT

1. Germination

1.1 Percentage germination

Control seeds (which were not exposed to red light or smoke treatment) from both the 1994 and 1995 seed lots had low germinability (Figs. 1-3). The seeds of the 1994 *P. taeda* and 1995 *P. elliottii* seeds had mean germination percentages of 12% and 15% respectively, while the 1994 *P. elliottii* seeds only 3 %.

Red light treatment stimulated germination in all seed lots, with the imbibed seeds responding better than the dry seeds (Figs. 1-3). In general this treatment improved germination by 50%.

Smoke treatment had variable effects, depending on the seed lot tested. Both 1994 seed lots showed enhanced germination when both dry and imbibed seeds were exposed to smoke (Figs. 1 and 2). The 1995 *P. elliottii* seeds, however, showed no response to smoke (Fig. 3).

Because limited numbers of 'damaged' seed were supplied, these seeds were exposed to red light treatment only, this treatment giving the most consistent improvement in germination. Percentage germination of these seeds however, was low (Fig. 4) and in contrast to the 1994 and 1995 seed lots, better germination occurred when dry seeds were exposed to the treatment. The moisture content of this seed batch was not determined. However, it is possible that the "dry" seeds were, in fact, at a higher moisture content than the 1994 and 1995 seed lots. The reason for the low response in imbibed seeds is not clear.

I) 1994 *P. taeda* seed lot

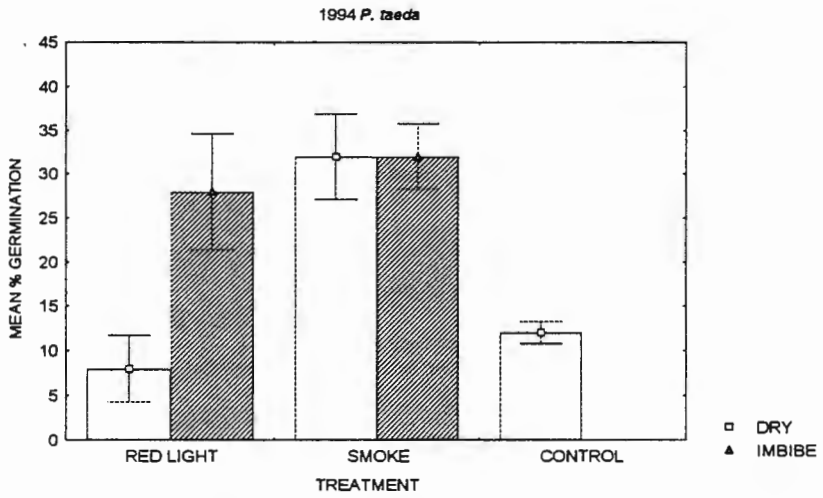

Figure1 : Percentage germination of the 1994 *P. taeda* seeds. The graphs show the mean ± the standard error.

II) 1994 *P. elliottii* seed lot

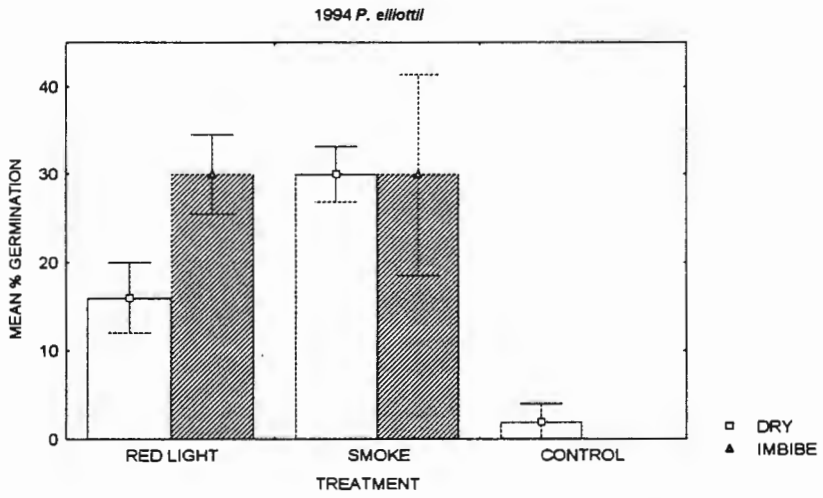

Figure 2: Percentage germination of the 1994 *P. elliottii* seeds, showing the mean and ± the standard error.

III) 1995 *P. elliottii* seed lot

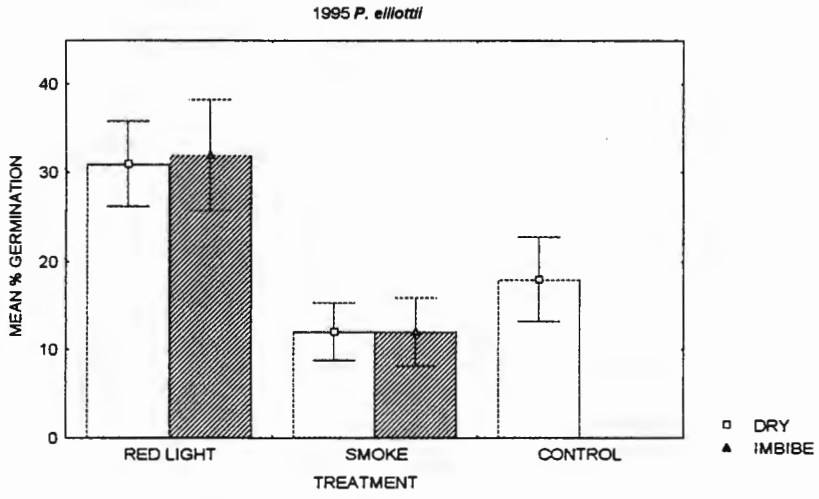

Figure 3 : Percentage germination of the 1995 *P. elliottii* seeds.

IV) 'Damaged seed lot

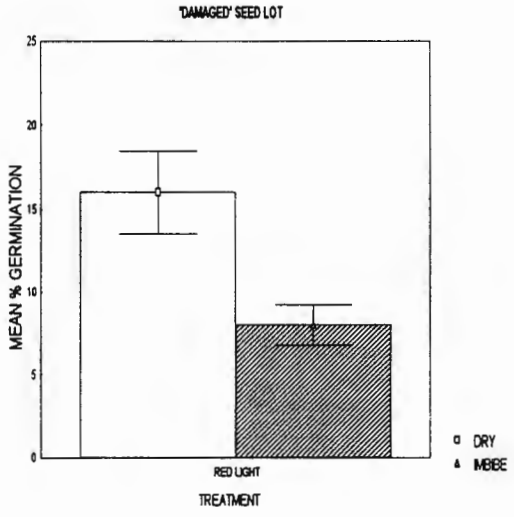

Figure 4 : 'Damaged' seeds exposed to red light treatment.

1.2. Germination rate

The germination rates of all the seed lots are presented in Table 1. The controls of the 1994 seeds showed low germination rates within each seed lot. However, the control of the 1995 *P. elliottii* seeds was high relative to the other seed lots. This implies that the 1995 seeds were more vigorous. The decline in vigour of the 1994 seed lot could have been due to their being older - which in turn could imply poor response to storage conditions.

It is evident that red light treatment caused an increase in the germination rate of the 1995 *P. elliottii* seeds, resulting in an increase in the both the dry and imbibed seeds by over 100% (relative to the other seed lots) (Table 1). The germination rate of the 'damaged' seeds were high relative to the 1994 seeds. This implies that the few seeds of the 'damaged' seed lot that did germinate (Fig. 4) were relatively vigorous. In general, the rates of the 1994 and 1995 imbibed treated seeds, were higher than the dry seeds, the opposite is true for the 'damaged' seeds.

Table 1 : Germination rate (Czabater, 1962) of the seed lots when exposed to different dormancy-breaking treatments, the data is presented as a mean ± standard deviation.

SEED LOT	DRY / IMBIBE	TREATMENT		
		RED LIGHT	SMOKE	CONTROL
1994 *P. taeda*	dry	0.05 ± 0.05	0.13 ± 0.03	0.11 ± 0.05
	imbibe	0.15 ± 0.09	0.2 ± 0	
1994 *P. elliottii*	dry	0.08 ± 0.02	0.2 ± 0	0.001
	imbibe	0.15 ± 0.08	0.3 ± 0.2	
1995 *P. elliottii*	dry	2.55 ± 0.03	0.99 ± 0.1	1.566 ± 0.01
	imbibe	3.31± 0.05	0.96± 0.01	
'damage' *P.elliottii*	dry	0.81± 0.02		
	imbibe	0.73 ± 0.01		

Smoke treated seeds of the 1995 *P. elliottii* seed lot also showed a higher germination rate than the other smoke treated seed lots. The germination rate of the 1994 *P. elliottii* seeds were higher than the 1994 *P. taeda* seeds. It is interesting that the smoke treatment of the 1995 *P. elliottii* seeds gave higher rates of germination than did the 1994 *P. taeda* and *P. elliottii* seed lots. This is opposite to the trend in the final percentage germination, in which the 1995 *P. elliottii* seeds had lower final percentage germination than the 1994 seed lots. This indicates that small percentage of seeds that did germinate, were vigorous.

2. CONDUCTIVITY

The conductivity readings (leakage) of the 1994 and 1995 seed lot controls were similar, but high relative to the dry seeds of the other treatments (Figs. 5-7).

When exposed to red light treatment, the dry seeds of 1994 and 1995 seed lots leaked more than the imbibed seeds (Figs. 5-7). This could account for the higher final percentage germination in the imbibed seeds (Figs. 1 and 2) and the higher germination rate (Table 1). The imbibed seeds of the 'damaged' seed lot leaked more than the dry seeds (Fig. 8) (which also corresponded to the germination data), with the leakage of the 'damaged' seed lot being the highest of all the seed lots. This could account for the low final percentage germination in this seed lot, the high germination rate, however indicates that there could have been a mixed bag of seeds, with the seeds that did germinate being vigorous, while most of the seeds were dead. The dry seeds of the 1995 *P. elliottii* seeds leaked almost double the amount of the imbibed seeds when exposed to red light treatment. This is interesting because the final germination percentage of the dry and imbibed seeds (Fig. 3) were similar.

Imbibed seeds exposed to smoke treatment, leaked more than the dry seeds in the 1994 and 1995 seed lots (Figs. 5-7) (although the significance was not calculated). It's interesting to note this because the dry and imbibed seeds of these seed lots showed equivalent final percentage germination (Figs. 1-3). Also, the germination rate of the

imbibed seeds was higher than the dry seeds (Table 1) (the significance was nit calculated). We are not certain what the reasons for this is, it could be due to experimental error.

I) 1994 *P. taeda* seeds

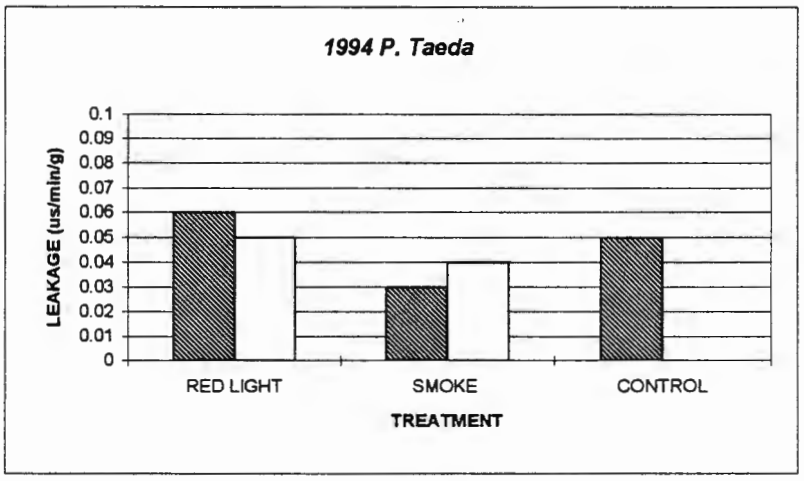

Figure 5 : Leakage of 1994 *P.taeda* seeds after each treatment.

II) 1994 *P. elliottii*

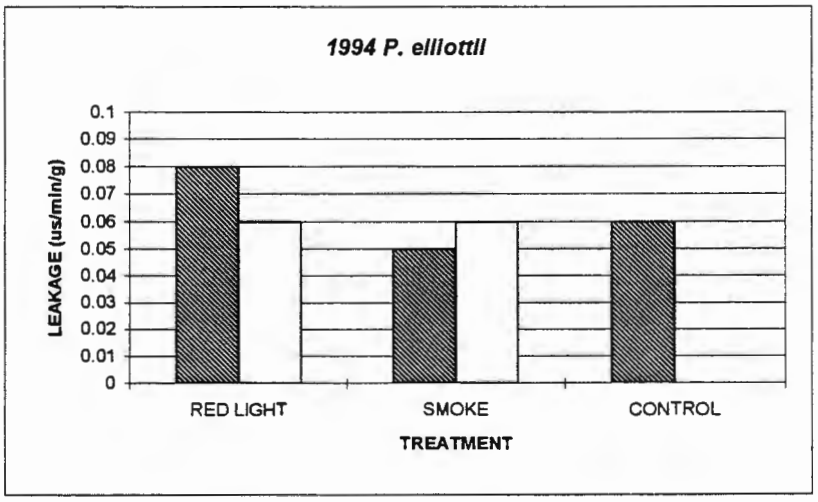

Figure 6 : Leakage of 1994 *P. elliottii* seeds after each treatment.

III) 1995 *P. elliottii*

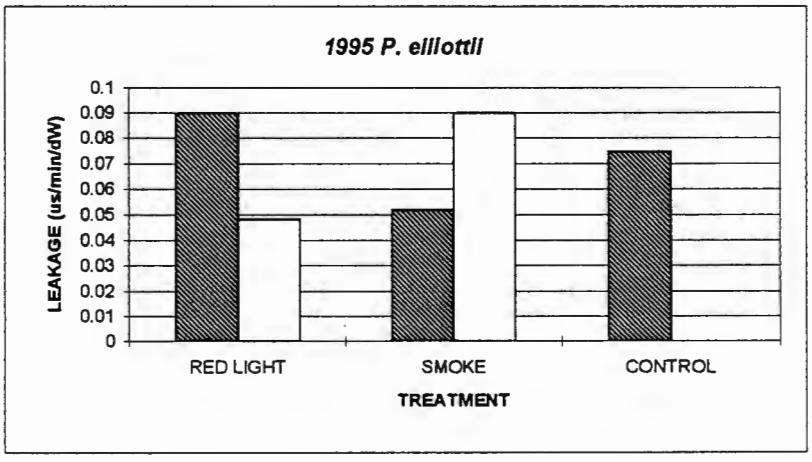

Figure 7 : Leakage of 1995 *P. elliottii* seeds before each treatment.

IV) 'Damaged' *P. elliottii*

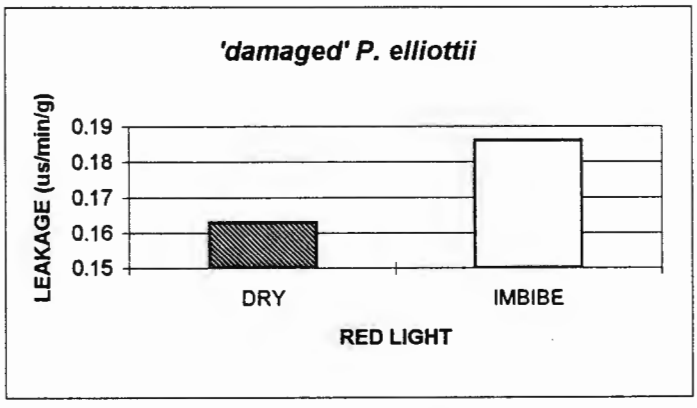

Figure 8 : Leakage of 'damaged' *P. elliottii* seeds before each treatment.

3. ELECTRON MICROSC0PY

One of the problems associated with the visualisation of the ultrastructural status of the seeds during this procedure is the potential of hydration of the seeds during the fixation procedure. As molecules of the fixative must be retained by the tissue they are fixing, a flow of water will precede the arrival of fixative solution to many cells, so that membranes may already be imbibed before they are fixed (Swift and Buttrose, 1972).With the application of these methods to the seeds, the possibility for changes in , or damage to cell ultrastructure, should be considered.

In the following micrographs, the ultrastructure of the dry and imbibed seeds of each seed lot will be observed. The focus will be to view differences between the protein bodies, vacuolation, lipid bodies and in some cases the nuclei and mitochondria (when visualised) between the dry and imbibed seeds of the different seed lots.

I) 1994 *P. taeda* seeds

a) DRY

Plate 1 shows the ultrastructure typical of dehydrated 1994 *P. taeda* seeds. Plate 1a shows the structure of the whole cell, with the tightly packed lipids and protein bodies Some protein bodies had electron transparent areas, (arrowed). Plate 1b shows severe lipid coagulation, which is the pooling of single lipid bodies to form larger lipid bodies (Smith and Berjak, 1995). Plate 1c shows a typical mitochondrion. These organelles were electron transparent with little internal structure (cristae were not well-defined). This is typical of inactive dry seed material.

b) IMBIBED

Plate 2 shows the ultrastructure of of a typical imbibed *P. taeda* seed. The lipid bodies are and the protein bodies have been used - which is typical of an imbibed seed (after 24 hours). Electron transparent areas are also seen. Plate 2b shows a mitochondrion which seems to be active, typical of an imbibed cell.

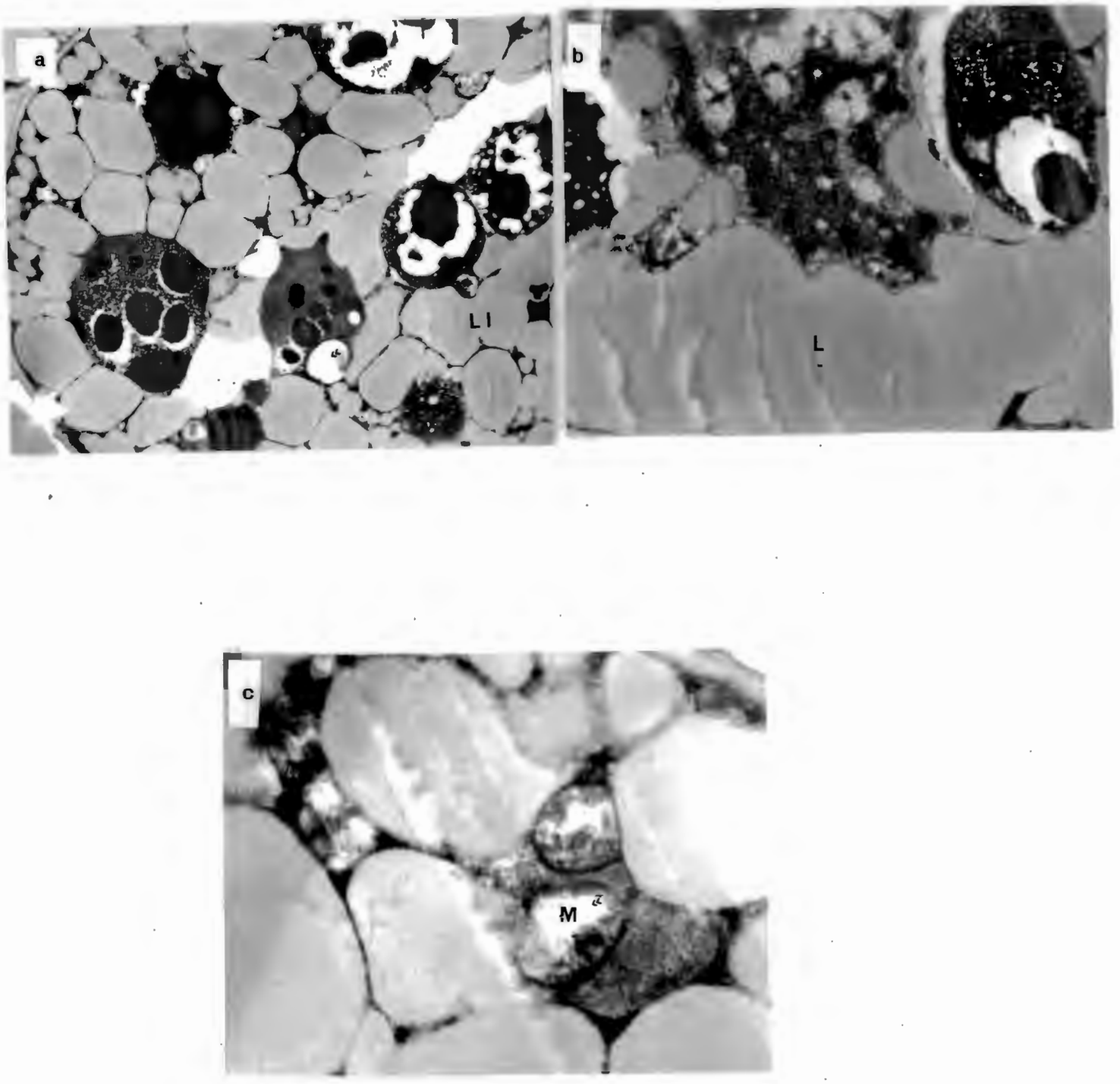

Plate 1 : Ultrastructural detail from the dry embryo of the 1994 *P. taeda* seeds. (a) protein bodies (**P**) ×6000, lipid bodies (**L**) ×6000; (b) lipid coagulation (**L**) ×8000; (c) mitochondrion with fine electron density (arrow) ×20000.

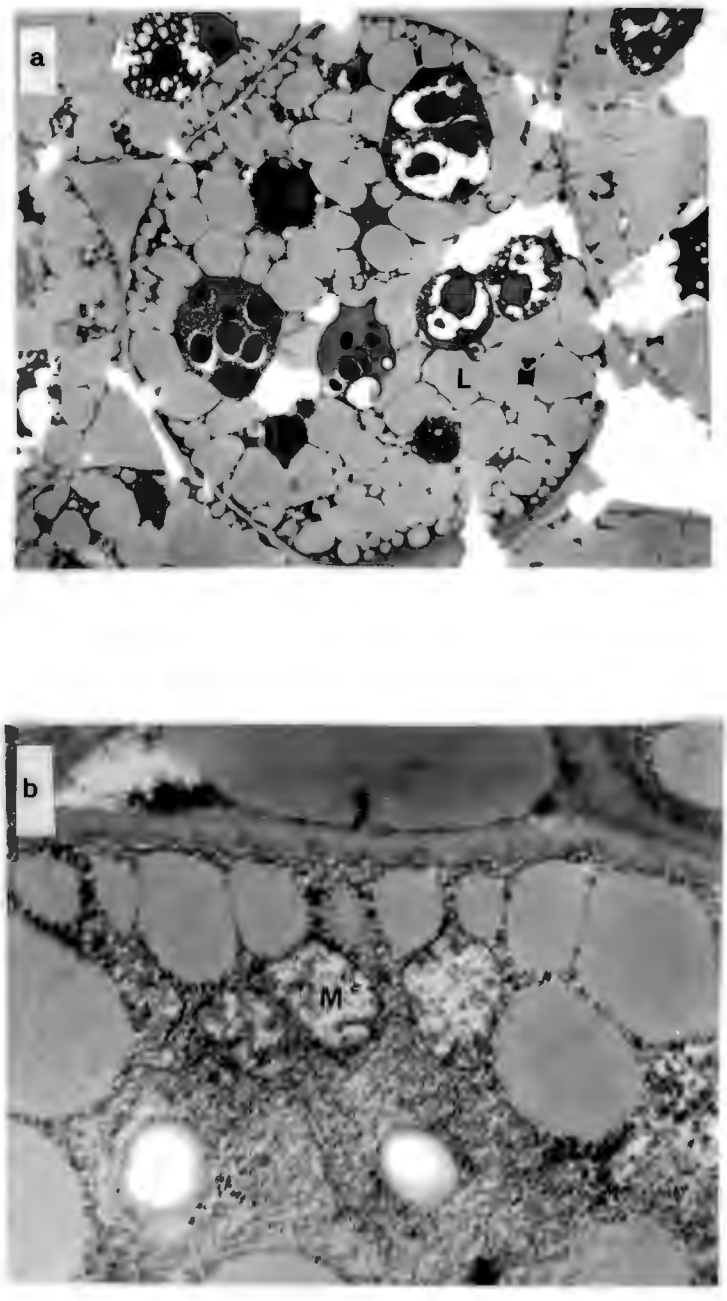

Plate 2 : Ultrastructural detail from the imbibed embryo of the 1994 *P. taeda* seeds. (a) Whole cell with protein bodies (**P**) ×6000, lipid bodies (**L**) ×6000 (b) mitochondrion (**M**) with fine electron density (arrow) ×20000.

I) 1994 *P. elliottii* seeds

a) DRY

Plate 3 shows the ultrastructure of a dry 1994 *P. elliottii* embryo. In Plate 3a the whole cell structure is shown, with the some protein bodies filled with dense electron dense material and others with elctron transparent areas. Full protein bodies indicate that the cell has accumulated reserves and the seed is in a state of desiccation tolerance. Empty protein bodies implies that the seed did not accumulate reserves at this stage of development. The lack of protein reserves could indicate that the seeds were harvested and dried at an immature stage, before reserve accumulation was completed. Plate 3b shows the lobed appearance, typical of nuclei in dry seeds. Chromatin is highly clumped - which is an indicator of damage . If our interpretation of an early harvest is correct, such damage could have been brought about by premature drying of the seed possibly, in a stage where it might have not reached full desiccation tolerance.

b) IMBIBED

Plate 4 shows the ultrastructure of an ebryo from 1994 *P. elliottii* embryo. Plate 4a shows the whole cell with protein bodies and lipid bodies, typical of an imbibed cell. Plate 3b shows an electron transparent mitochondrion and plastids or unfilled vacuoles. The protein bodies are not expected to be empty 24 hours after imbibition, this is because the seeds will generally use its sugars before mobilizing all its proteins. Once again, empty protein bodies are indicative of immature seeds, harvested before desiccation tolerance was reached.

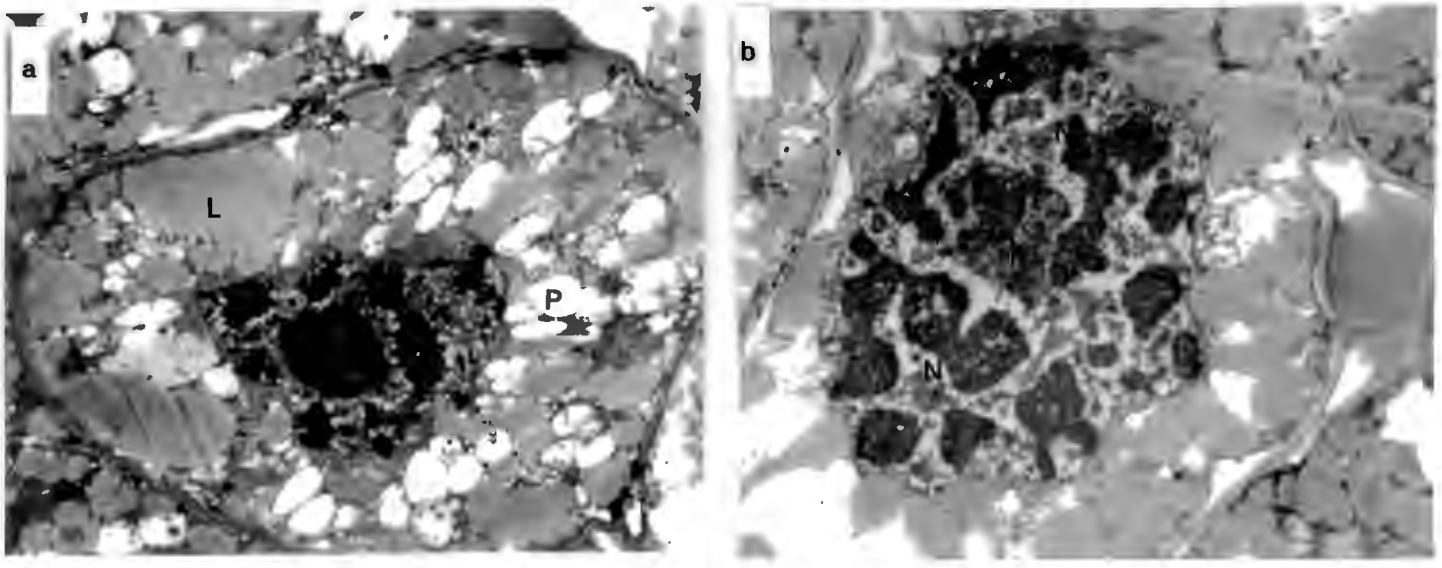

Plate 3 : Ultrastructural detail from the dry embryo of the 1994 *P. elliottii* seeds. (a) lipid bodies ×6000 (**L**), protein bodies (**P**) with electron transparent areas (arrowed) ×6000 (b) lobed nucleus (**N**) ×15000.

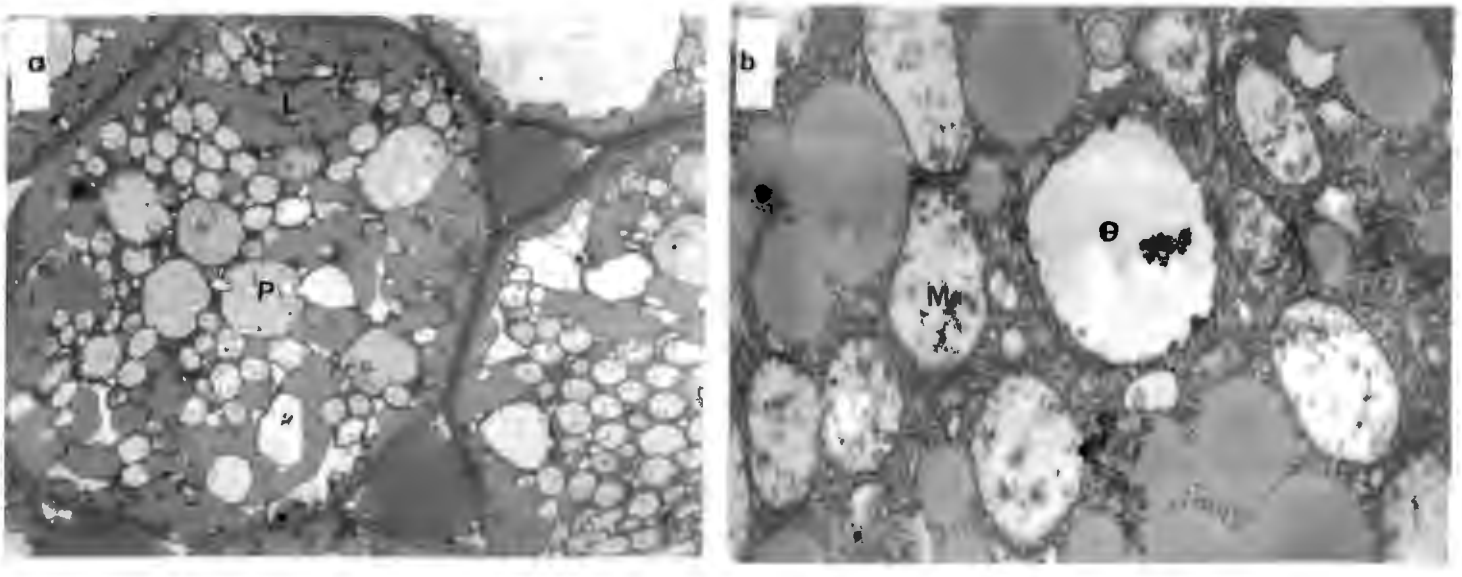

Plate 4 : Ultrastructural detail from the imbibed embryo of the 1994 *P. elliottii* seeds. (a) lipids (**L**) ×6000, protein bodies with electron transparent areas (arrowed) ×6000. (b) mitochondrion (**M**) ×20000; plastid or unfilled vacuole (**O**).

II) 1995 *P. elliottii* seeds

a) **DRY**

Plate 5 shows a intact cell structure from an embryo of the 1995 *P. elliottii* dry seed. Plate 5a shows the nature of protein bodies and lipid bodies, a loss of electron density indicates cell damage. Figure 5b shows electron transparent plastids containing starch containing and lipid bodies, which are small and clumped, indicative of a dry cell.

b) **IMBIBED**

Plate 6a shows the intact cell structure from an embryo of aa imbibed 1995 *P. elliottii* seed. Electron transparent vacuoles were evident. No protein bodies were present, implying either protein utilisation during imbibition or lack of protein in the first instance. As some dry seeds seeds have more protein, the second interpretation is more feasible. The clumped nucleus is indicative of cell damage. Plate 6b shows a typical mitochondrion, with few cristae and lipid bodies.

III) 'Damaged' *P. elliottii* seeds

a) **DRY**

Plate 7 shows details of the a dry 'damaged' *P. elliottii* embryos. There is extensive lipid coagulation and many electron transparent organelles. These could be unfilled protein bodies or a plastid, a few starch grains are evident. Plate 7b shows the electron transparent protein bodies and lipid coagulation in more detail.

b) **IMBIBED**

Numerous electron transparent organelles were present (Plate 8). This could be empty protein bodies. Electron dense deposits occurred at the periphery of these organelles , this is typical of early stages of protein accumulation and it is possible that the seeds were harvested before this process was completed.

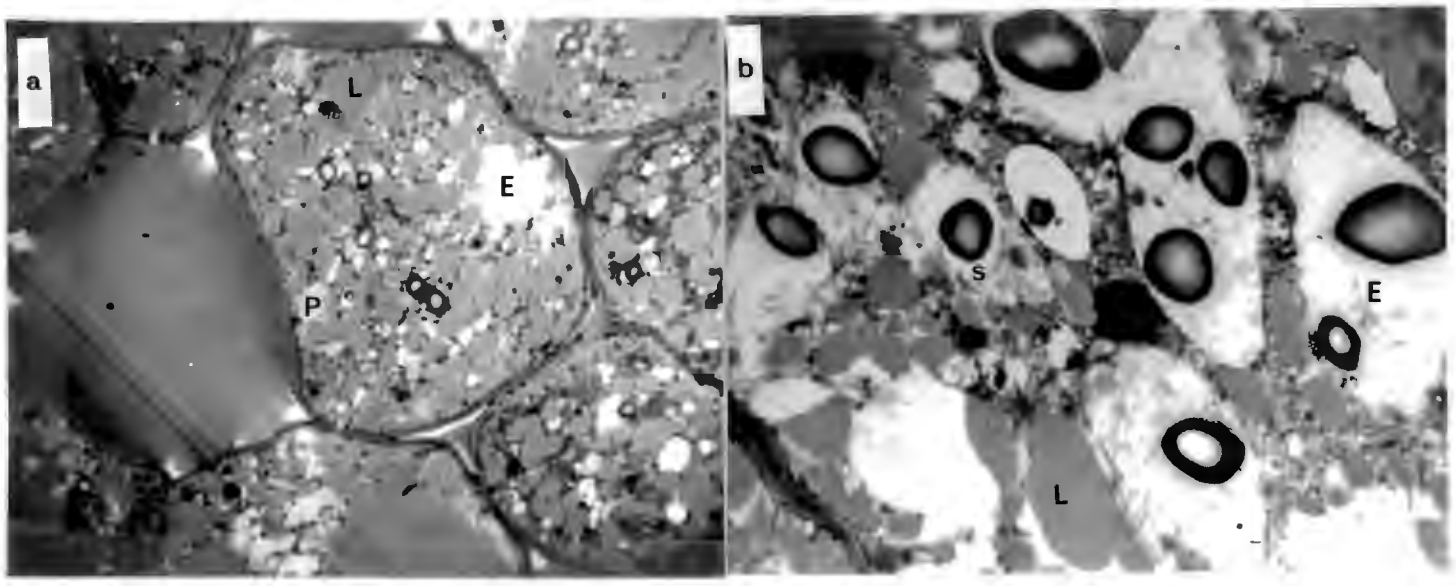

Plate 5 : Ultrastructural detail from the dry embryo of the 1995 *P. elliottii* seeds. (a) whole cell lipid bodies (**L**) ×2000, electron transparent plastid with starch (**E**) ×2000, electron transparent protein bodies (**P**). (b) lipid bodies (**L**) ×50000, electron transparent plastid with starch grain (**s**) ×50000 , electron transparent vacoule (**E**) ×50000.

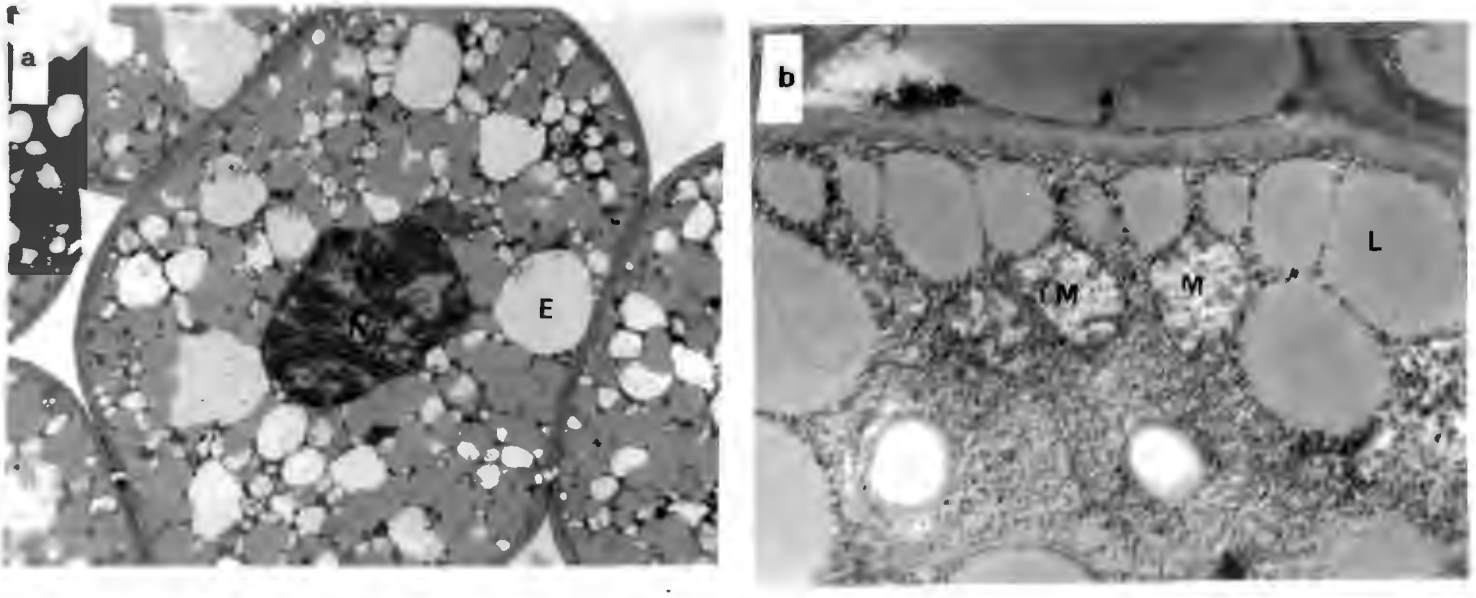

Plate 6 : Ultrastructural detail from the imbibed embryo of the 1995 *P. elliottii* seeds. (a) whole cell with electron transparent vacuole (**E**) ×3700, clumped nucleus (**N**) ×3700 (b) mitochondrion (**M**) with cristae ×15000, lipid bodies (**L**) ×15000.

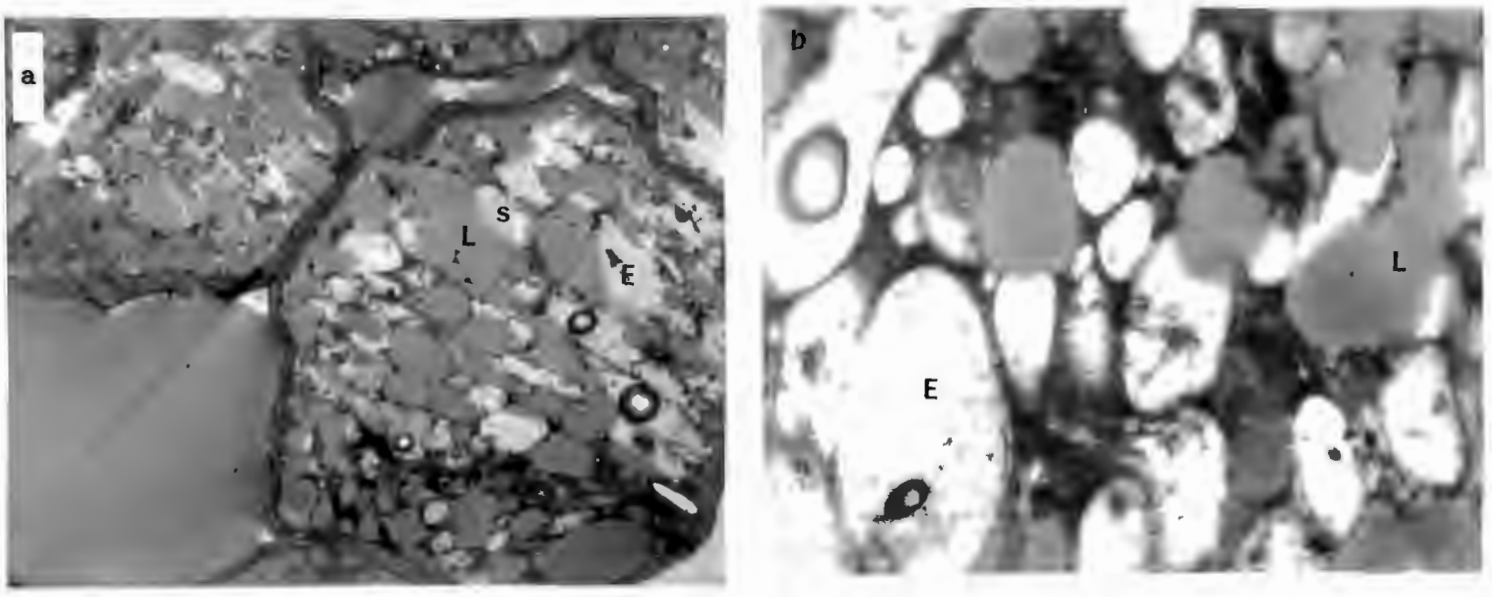

Plate 7 : Ultrastructural detail from the dry embryo of the 'damaged' *P. elliottii* seeds. (a) whole cell with lipid bodies (**L**) ×3000, electron transparent organelles (**E**) ×3000, electron transparent organelle containing starch (s) ×3000,(b) electron transparent organelle containing starch (**E**) ×15000, lipid bodies (**L**) ×15000.

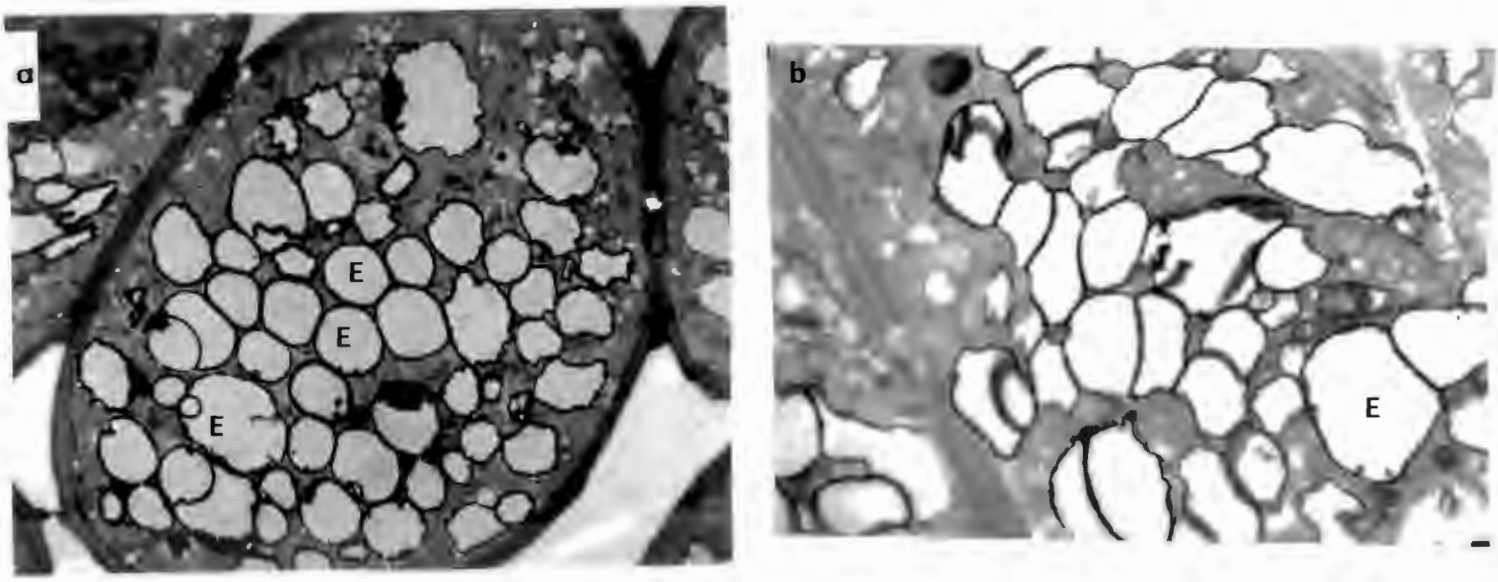

Plate 8 : Ultrastructural detail from the imbibed embryo of the 'damaged' *P. elliottii* seeds. (a) numerous electron transparent organelles (**E**) ×4500, (b) elecron transparent organelle (**E**). ×15000.

4. TISSUE CULTURE

I) 1995 *P. elliottii* seed lot

The 1995 *P. elliottii* seeds grown in different hormone treatments, showed a response when the hormones BAP + IAA, were included in the medium (Fig. 1).

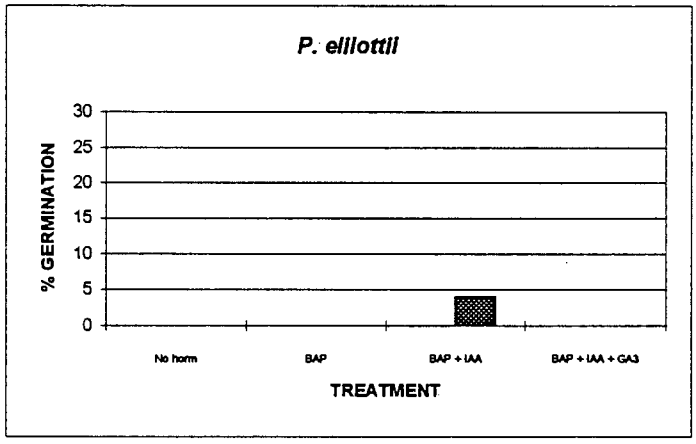

Figure 9 : Percentage germination of the 1995 *P. elliottii* seeds treated with different hormones.

II) 'damaged' *P. elliottii* seed lot

The 'damaged' seeds treated with different hormones only responded to BAP and BAP + IAA, but the percentage germination was very small (Fig. 10).

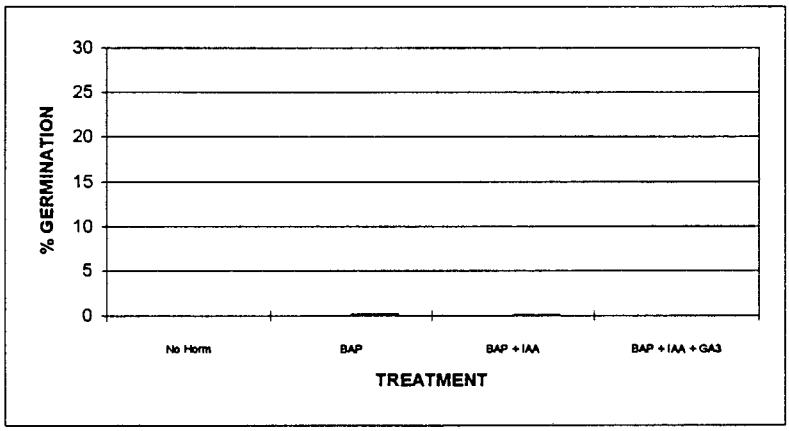

Figure 10 : Percentage germination of the 'damaged' *P. elliottii* seeds treated with different hormones.

DISCUSSION

In the preliminary experiments performed on the 1994 *P. taeda* and *P. elliottii* seeds, excessive fungal activity was observed. In order to eliminate fungal infection as a factor affecting the germination results, it was decided to treat the 1995 *P. elliottii* seeds (*P. taeda* was not tested due to seed shortage) with benlate (a commercial fungicide). The observed fungal infection was decreased considerably. The excessive observed fungal infection of the *P. elliottii* seed lot could be a factor which contributed to the non-responsiveness of this seed lot to the control treatment (high humidity, heat (30°C) and dark). Possibly the increase in fungal infection of these seeds is due to sub-optimal storage conditions in the MONDI nurseries. A seed is a nutrient source for fungal pathogens (Mycock and Berjak, 1995) and if not stored under suboptimal storage conditions, could be exposed to both internally and externally located fungi throughout its existence. Furthermore, the succession of the storage fungi is controlled by seed moisture content and temperature (Agarwal and Sinclair, 1987). Thus, the exposure of the seeds to high humidity and heat, could have stimulated the succession of storage fungi, resulting in the low germinability (Figs. 1-3) and germination rates in the controls (Figs. 5-7). In contrast, Blakeway, (pers comm), found that this treatment stimulated germination in seeds.

Although smoke and red light treatment stimulated the percentage germination in both the species studied, some of the seeds did not respond to these treatments. Some of the seeds probably did not respond due to seed damage caused by the exposure to pre-treatments (e.g. surface sterilisation and benlate). For example, smoke treatment caused an increase in the percentage germination of the 1994 seed lots, but did not cause such a high response in the 1995 seed lots which was treated with benlate. Also, the high leakage of some of the seed lots (Figs.5-8) confirms that a proportion of the seeds were damaged. However, the proportion of non-germinable seeds, probably did not germinate due seed damage, not only due to pre-treatments but due to other factors.

Ultrastructural studies confirmed a certain degree of damage in lipid coagulation and chromatin clumping (Smith and Berjak, 1995). These studies also reveal incomplete

reserve accumulation in many dry seeds. When shed from the parent plant, a seed is desiccation tolerant and has maximal accumulation of reserves to support the germination process (Bewley and Black, 1994). The acquisition of desiccation tolerance i.e. the stages of development leading up to drying below a moisture content of 20%, is critical in seed development, since it determines the vigour and viability of the seeds (Bewley and Black, 1994). Kermode and Bewley (1985) found that seeds of the castor bean (*Ricinus communis*) did not germinate before desiccation tolerance was reached. Furthermore, they found that the ability of seeds to tolerate desiccation improves progressively with time, probably because of physiological and morphological changes which take place as seed development proceeds - which produces protective substances in later stages (Bewley and Black, 1994, Farrant and Vertucci, 1995). From our results it would appear that the 1994 and 1995 *P. elliottii* seed lots and the 'damaged' seed lots have been harvested prematurely - before reserve accumulation was complete. Desiccation at this premature stage could have caused considerable damage and resulted in the low viability (Figs. 1-4, Plates 3-8). The variability in germination data could have arisen from harvesting seeds with variable developmental maturity. More mature seeds, with an adequate supply of reserves would have survived subsequent desiccation and have sufficient reserves to complete germination. Very immature seeds, on the other hand, would have suffered considerable damage and not have sufficient reserves to germinate. Premature harvest could have induced a slight dormancy in some of the more mature seeds - alternatively, their treatments could have facilitated some degree of after-ripening in the seeds (Bewley and Black, 1994), which improved their germinability. The *P. taeda* seeds, however, showed little seed damage and only a small degree of incomplete reserve accumulation (Plate 1 and 2), hence the higher percentage germination (Figs. 1) and lower leakage results (Fig. 5). But, these results cannot be confirmed due to the small sample size of *P. taeda* that was studied.

Finally, the treatment of the seeds with hormones had little or no effect on the germinability of the seeds (Figs. 9-10). This could be due to experimental error or the seeds not responding to the selection of hormones we applied, further experimentation is required.

CONCLUSIONS

There are several reasons for the factors that could contribute toward the problem MONDI is experiencing with the low germinability of *P. taeda* and *P. elliottii* seeds. From our evidence, we conclude that the following could be the reasons for the low germinability :

Seeds could be dormant, due to their positive response to red light and smoke treatment. However, if the seeds are not dormant, they could be damaged due to :

a) The seeds being immature, i.e. the seeds being picked too early and not accumulating enough reserves to reach their full germination potenetial.

b) Postharvest practices are not allowing the seeds to reach desiccation tolerance, which is a critical factor determining the vigour and viability of the seeds.

c) Internal and external fungal infection (which could be enhanced by sub-optimal storage practices) could be resulting in utilisation of the seeds reserves and thus resulting in tissue damage.

In all the above cases, further experimentation is required.

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CITED REFERENCES

Agarwal V.K. and **Sinclair J.B**. (1987). Principles of seed Pathology, vols. 1 and 2 CRC Press, Boca Raton, FL.

Ammer, S., Benbadis, A. and **Tripathi, B.K.** (1987). Floral induction in date palm seedling (*Phoenix dacttylifera* var *Deglet Nour*) cultured *in vitro. Canadian Journal of Botany* **65 :** 137-142.

Bayley, A. and **Rosser, K.** (1992) unpublished. An investigation of the poor emergence of *P.patula* and *P. elliottii* seed in the SAPPI nurseries. SAPPI research project propogation programme.

Bewley, J.D. (1986). Membrane changes in seeds as related to germination and perturbations resulting from deterioration in storage. *In* (eds. M.B. McDonald and C.J. Nelson) *Physiology of Seed Deterioration.* CSSA Special Publication. No. 11. Crop Science Society of America, Inc. Madison, Wisconsin, USA.

Bewley, J.D. and **Black, M.** (1994). *Seeds, Physiology of Development and Germination.* 2nd ed. Plenum Press. New York

Brown, N. A. C. (1993). Promotion of germination of fynbos seeds by plant-derived smoke. *New Phytologist* **123 :** 575-583.

Christensen, C.M. and **Kaufmann, H.H.** (1974). Microflora. *In* (eds. C.M. Christensen) *Storage of Cereal Grains and their Products,* 158-191. American association of Cereal Chemists Inc., St. Paul, MN.

Czabater, F.J. (1962). Germination Value : an index combining speed and completeness in germination of pine seeds. *Forest Science* **8**(4) : 386 -365.

Dixon, K.W. , Roche, S. and **Pate, J.S.** (1995). The promotive effect smoke from burnt native vegetation on seed germination of Western Australian plants. *Oecologia* **101 :** 185-192.

Farrant, J.M. and **Vertucci, C.** (1995). The Acquisition and Loss of Desiccation Tolerance. *In* J. Kigel and G. Galili (eds.) *Seed Development and Germination.* Marcel Dekker Inc. New York.

Hart, J. W. (1988). *Light and Plant Growth.* Unwin Hyman. Boston.

Hilhorst, H.W.M. and **Karssen, C.M.** (1988). Dual effect of light on the Gibberellin- and Nitrate-Stimulated seed germination of *Sisymbrium officinale* and *Arabidopsis thaliana. Plant Physiol.* **86 :** 591-597.

Hilhorst, H.W.M. (1993). New Aspects of seed dormancy. *Proceedings of the Fourth International Workshop on Seeds. Basic and Applied Aspects of Seed Biology. Angers, France,* 20-24 July, 1992, Vol. 2 (eds. D. Come and F. Corbineau), pp. 571-579. Universite Pierre et Marie Curie.

Kermode, A.R. and **Bewley, J.D.** (1985). The role of maturation drying in the transition from seed development to germination. I. Acquisition of desiccation tolerance and germinability in *Ricinus communis* L. seeds. *J. Exp. Bot.* **36 :** 1906-1915.

Murashige, T. and **Skoog, F.** (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiologia plantarum* **15 :** 493-497.

Mycock, D.J. and **Berjak, P.** (1992). Paradoxical behaviour of seed storage and field fungi : an overview. *South African Journal of Science* **88 :** 371-375.

Mycock, D.J. and **Berjak, P.** (1995). The implications of seed-associated mycoflora during storage. *In* J. Kigel and G. Galili (eds.) *Seed Development and Germination.* Marcel Dekker Inc. New York.

Parrish and **Leopold** (1978). Light and seed germination. Academic Press.

Roberts (1973). Predicting seed longevity. *Seed Sci. Technology* **1 :** 499-514.

Simon, E.W. (1978). Membranes in dry and imbibing seeds. *In* J.H. Crowe and J.S. Clegg (eds.) *Dry Biological Systems.* Academic Press, New York.

Scorza, R. (1982). *In Vitro* Flowering. *Hort. Review* **4 :** 106-127.

Smith, M.T. and **Berjak, P.** (1995). Deteriorative changes associated with loss of viability of stored seeds. *In* J. Kigel and G. Galili (eds.) *Seed Development* and *Germination.* Marcel Dekker Inc. New York.

Swift, J.G. and **Buttrose, M.S.** (1972). Ultrastructure. *J. ultrastruc. Res.* **40 :** 378.

van Staden, J.; Drewers, F.E. and **Jager, A.K.** (1995a). The search for germination s stimulants in plant-derived smoke extracts. *S.A. J. of Botany* **61 :** 260-263.

Vleeshouwers, L.M.; Boumeester, H.J. and **Karrsen, C.M.** (1995). Redefining seed dormancy : an attempt to integrate physiology and ecology. *J. of Ecology* **83 :** 1031-1037.